# Vitamin C content of rotifers enriched with the microalga *Isochrysis* aff. *galbana* (T-iso) collected from different growth conditions.

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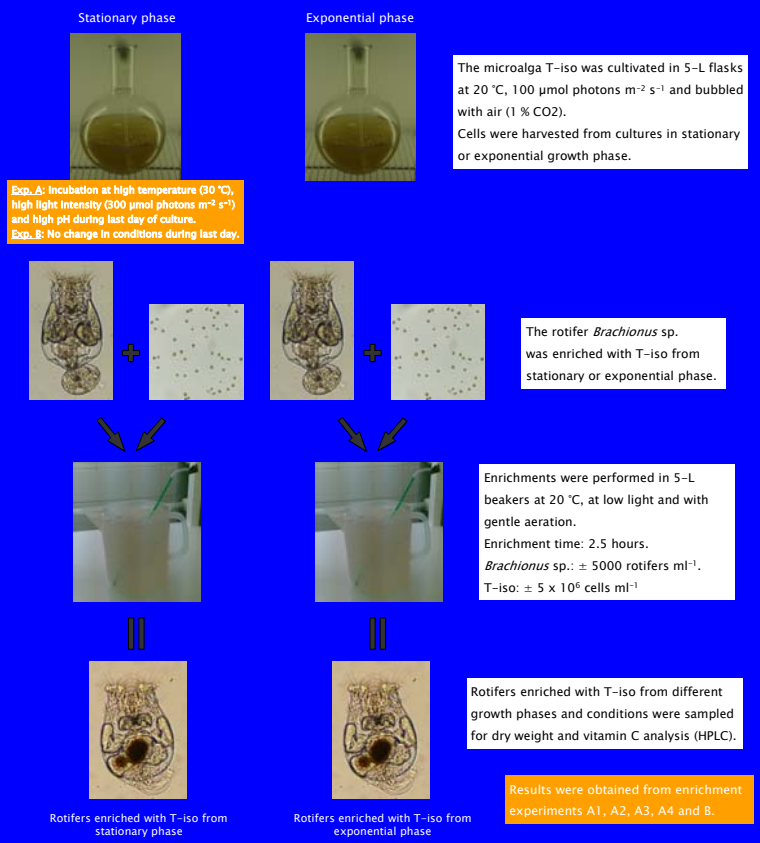
## Introduction

- *Isochrysis* aff. *galbana* (Clone T-iso) is among the most popular microalgal species used to enrich rotifers of the genus *Brachionus*.
- *Brachionus* is an important first feed during the initial stages of several marine fish species in aquaculture.
- T-iso is rich in the hydrosoluble vitamin ascorbic acid (vitamin C) which is an essential vitamin in fishes.
- Vitamin C content of T-iso has been found to vary with growth phase and light intensity.

The aim of our study was to evaluate if different vitamin C contents in T-iso from different growth phases and conditions would lead to correspondingly different vitamin C contents in rotifers enriched with these microalgae.

## Materials and methods




The microalga T-iso was cultivated in 5-L flasks at 20 °C, 100 µmol photons $m^{-2}$ $s^{-1}$ and bubbled with air (1 % CO2).
Cells were harvested from cultures in stationary or exponential growth phase.

The rotifer *Brachionus* sp. was enriched with T-iso from stationary or exponential phase.

Enrichments were performed in 5-L beakers at 20 °C, at low light and with gentle aeration.
Enrichment time: 2.5 hours.
*Brachionus* sp.: ± 5000 rotifers $ml^{-1}$.
T-iso: ± 5 x $10^6$ cells $ml^{-1}$

Rotifers enriched with T-iso from different growth phases and conditions were sampled for dry weight and vitamin C analysis (HPLC).

Results were obtained from enrichment experiments A1, A2, A3, A4 and B.

## Results

Cultures of T-iso in exponential phase all showed a higher vitamin C content than cultures in stationary phase. They also showed variation in dry weight (DW) content, with an especially high DW content of the culture in stationary phase of experiment B (Table 1).

**Table 1**

| | Vit. C content T-iso (µg (mg DW)$^{-1}$) | | Vit. C content T-iso (fg cell$^{-1}$) | | DW content T-iso (pg cell$^{-1}$) | |
|---|---|---|---|---|---|---|
| Experiment | Stationary | Exponential | Stationary | Exponential | Stationary | Exponential |
| A1 | 1.37 | 4.30 | 25.17 | 125.12 | 19.47 | 29.07 |
| A2 | 1.70 | 3.97 | 61.47 | 75.89 | 36.08 | 19.12 |
| A3 | 1.60 | 3.66 | 42.07 | 87.97 | 26.33 | 24.04 |
| A4 | 1.95 | 2.69 | 54.49 | 84.07 | 27.39 | 31.23 |
| B | 2.50 | 3.19 | 128.81 | 88.64 | 50.82 | 27.82 |

However, only in two experiments vitamin C content was higher in rotifers enriched with T-iso from exponential phase than in rotifers enriched with T-iso from stationary phase (Table 2).

**Table 2**

| | Vit. C content *Brachionus* sp. (µg (mg DW)$^{-1}$) | | Vit. C content *Brachionus* sp. (pg rotifer$^{-1}$) | | DW content *Brachionus* sp. (ng rotifer$^{-1}$) | |
|---|---|---|---|---|---|---|
| Experiment | Stationary | Exponential | Stationary | Exponential | Stationary | Exponential |
| A1 | 0.47 | 0.91 | 94.16 | 177.47 | 199.29 | 195.21 |
| A2 | 0.76 | 0.76 | 159.22 | 144.24 | 208.73 | 190.72 |
| A3 | 0.92 | 0.86 | 133.55 | 177.14 | 144.42 | 206.04 |
| A4 | 0.68 | 0.79 | 122.27 | 141.11 | 181.04 | 178.65 |
| B | 1.16 | 1.05 | 210.39 | 181.53 | 181.96 | 172.81 |

Based on vitamin C and DW content of the microalgae and on rotifer numbers in the enrichments, the amount of DW and vitamin C added to the rotifers was calculated (Table 3). Although vitamin C content of T-iso cultures in exponential phase was higher, rotifers enriched with these cultures did not always receive a higher amount of vitamin C.

**Table 3**

| | DW (T-iso) added per $10^6$ rotifers (mg) | | Vit. C (T-iso) added per $10^6$ rotifers (µg) | |
|---|---|---|---|---|
| Experiment | Stationary | Exponential | Stationary | Exponential |
| A1 | 16.44 | 35.42 | 22.47 | 152.47 |
| A2 | 37.24 | 16.53 | 63.44 | 65.63 |
| A3 | 29.68 | 19.19 | 47.43 | 70.22 |
| A4 | 31.16 | 32.89 | 60.89 | 88.54 |
| B | 63.38 | 31.03 | 158.55 | 98.86 |

We estimated ingestion rates of the rotifers (Table 4). Ingestion rates increase with increasing DW:rotifer ratio (Table 3).

**Table 4**

| | Ingestion rate of Brachionus sp. (mg DW ($10^6$ rotifers)$^{-1}$ h$^{-1}$ | |
|---|---|---|
| Experiment | Stationary | Exponential |
| A1 | 5.02 | 10.81 |
| A2 | 11.37 | 5.05 |
| A3 | 9.06 | 5.86 |
| A4 | 9.51 | 10.04 |
| B | 18.4 | 11.16 |

## Discussion

The enrichment experiments were set up in such a way that in all enrichments rotifers received the same amount of microalgae. However, variation in DW content of T-iso and in rotifer numbers led to variation of the DW: rotifer ratios in the enrichments between 16 and 63 mg DW per $10^6$ rotifers (Table 3). This variation influenced the actual amount of vitamin C added per rotifer and affected the ingestion rate of the rotifers.

This explains why in some cases rotifers enriched with T-iso from stationary phase, with lower vitamin C content, had a higher vitamin C content than rotifers enriched with T-iso from expontential phase, with higher vitamin C content.

## Conclusion

The vitamin C content of rotifers can be manipulated by enrichment with cultures of T-iso with varying vitamin C content, obtained by varying growth conditions.
However, special care should be taken to ensure that the ratio between microalgal dry weight and rotifer numbers in the enrichments does not vary much or is above the ratio where maximum ingestion rate of the rotifers is reached.

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